Visualization of DNA double-strand breaks induced by high LET particles and X-rays in murine bones and soft tissues*

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DNA double strand breaks (DSBs) are the most lethal and cytotoxic type of DNA damage. DSBs can be induced by exogenous agents, such as ionizing radiation (IR), or by normal cellular processes, such as V(D)J recombination. DSBs generated by high LET radiation are more complex than low LET DSBs and generally more difficult to repair [1].

As part of the GREWIS project, this study is aimed at the biodosimetric measurement of inhaled radon gas in different murine tissues. Radon (Rn-222) is a radioactive noble gas; during its radioactive decay three biologically relevant α-particles are emitted. The goal of this project is to detect α-particle induced DSB tracks in murine tissues to reveal the diffusion patterns of radon gas inside the body and any potential accumulation in specific organs.

Radon gas is used for its therapeutic effects in radon therapy caves in Bad Gastein and Bad Kreuznach [2]. The main indications for therapeutic radon treatment include chronic inflammatory diseases and degenerative joint diseases, such as rheumatoid arthritis. Due to its long-lasting effects, radon therapy provides an alternative treatment option that often allows patients to stop taking other pain medications [3].

Prior to *in vivo* radon experiments, wild type C57BL/6 mice were irradiated with low fluence carbon ions at the GSI particle accelerator. This experiment was used to evaluate the staining efficacy of heavy ion induced DSBs in different murine tissues. Using paraffin embedded tissue sections, 53BP1 foci tracks after carbon ion irradiation were successfully visualized and quantified in murine lung, heart, liver, intestine, kidney, spleen, and brain tissues (Fig. 1 A). As radon therapy is predominantly prescribed for chronic inflammatory diseases of the musculoskeletal system, the visualization of radon induced DNA damage in bone tissue is central to this project. Therefore, efforts were focused on establishing a protocol for immunofluorescence staining in bone tissue. After formaldehyde fixation, bone tissue (*in vivo* irradiation with 10 mGy X-rays) was decalcified for 24 hours using an EDTA solution. The samples were subsequently embedded in paraffin and cut into 4μm sections using a microtome. Using γH2AX and 53BP1 antibodies, DNA damage was visualized in the compact bone, periosteum and bone marrow (Fig. 1 B).

Two animal experiments (radon concentrations: 440 kBq/m^3, 40 kBq/m^3) were recently conducted. C57BL/6 mice were exposed to radon for one hour and organs were subsequently harvested after 15 minutes, 24 hours and 7 days. Hence, future efforts will focus on the analysis of *in vivo* radon experiments.

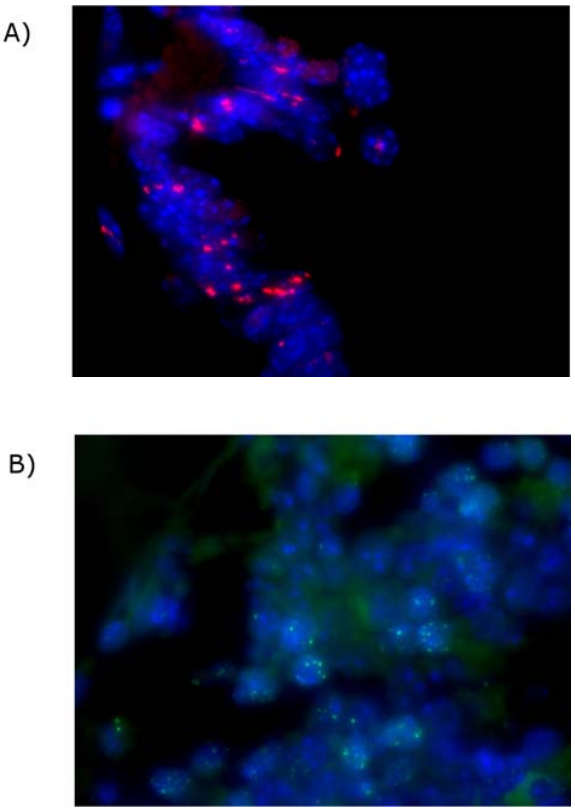

Figure 1: Visualization of DNA DSBs: (A) 53BP1 foci in murine lung tissue cells after carbon ion irradiation. (B) γH2AX foci in murine bone marrow after X-ray irradiation.

References

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